Monitoring glyphosate residues in transgenic glyphosate-resistant soybean

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Abstract: The availability of Roundup Ready (RR) varieties of soybean has increased the use of glyphosate for weed control in Argentina. Glyphosate [(N-phosphonomethyl)glycine] is employed for the eradication of previous crop vegetation and for weed control during the soybean growing cycle. Its action is effective, and low environmental impact has been reported so far. No residues have been observed in soil or water, either of glyphosate or its metabolite, AMPA (aminomethylphosphonic acid). The objective of this work was to monitor glyphosate and AMPA residues in soybean plants and grains in field crops in Santa Fe Province, Argentina. Five sites were monitored in 1997, 1998 and 1999. Individual soybean plants were sampled from emergence to harvest, dried and ground. Analysis consisted in residue extraction with organic solvents and buffers, agitation, centrifugation, clean-up and HPLC with UV detection. In soybean leaves and stems, glyphosate residues ranged from 1.9 to 4.4 mg kg\(^{-1}\) and from 0.1 to 1.8 mg kg\(^{-1}\) in grains. Higher concentrations were detected when glyphosate was sprayed several times during the crop cycle, and when treatments approached the flowering stage. AMPA residues were also detected in leaves and in grains, indicating metabolism of the herbicide.

Keywords: glyphosate residues; glyphosate-resistant soybean

1 INTRODUCTION

Glyphosate [(N-phosphonomethyl)glycine] is a highly effective broad-spectrum herbicide. The isopropylamine salt of glyphosate is used as a non-selective, non-residual, broad-spectrum, foliar applied, post-emergence herbicide. After application, glyphosate is absorbed by the foliage and translocated throughout stems, leaves and roots of the entire plant. Its inhibitory effect in susceptible plants is based on binding to the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS) in the biosynthetic pathway of aromatic amino acids.1

Using known mechanisms of tolerance in plants2 and resistant bacteria3 it has been possible to produce herbicide-resistant crops by recombinant DNA technology.4 Glyphosate-tolerant transgenic soybeans were obtained by transferring genes of an EPSPS variant with decreased sensitivity to glyphosate and with no or very little loss of catalytic efficiency. These mutant genes provided high levels of glyphosate resistance.5 In Argentina, these soybean varieties (Roundup Ready; RR) were introduced in 1996.6 In 1999, 80% of the cropped surface was seeded with these varieties, thus allowing excellent control of annual and perennial species of weed by the use of glyphosate.7

The development of crop cultivars with resistance to selected herbicides has the potential to impact environmental quality, food safety, consumers and crop producers in either a positive or a negative manner.8 The primary concerns are usually the feasibility of controlling the volunteer transgenic crop and the opportunity for indiscriminate introgression of the herbicide-tolerance gene into agricultural and natural ecosystems.9

The metabolism of glyphosate has been extensively investigated both in the environment and under laboratory conditions. Microbial action is responsible for the degradation of the herbicide in the soil, and aminomethylphosphonic acid (AMPA) is the major metabolite detected.10 Plants have been reported not to metabolise glyphosate. However, glyphosate and AMPA residues were detected in cell cultures of soybean,11 in strawberry plants and fruits12 and in upper crown foliage in forests.13 No published information is available of glyphosate and AMPA residues in glyphosate-resistant (RR) soybean crops.
The objective of the present study was to monitor RR soybean farm plots to determine glyphosate and AMPA residues in plants and grains following glyphosate treatment of established crops.

2 MATERIALS AND METHODS

2.1 Study sites

Established RR soybean was monitored at three farms in 1997, at one in 1998 and at another in 1999. These sites were located in Frank, Las Colonias Department, Santa Fe Province, Argentina. All sites had the same soil type and similar soybean management. Glyphosate-isopropylamine 480 g AE litre\(^{-1}\) SL (Roundup) was applied at different rates each year: 1.2 kg AE ha\(^{-1}\) in 1997, 0.96 kg AE ha\(^{-1}\) in 1998 and 1.68 kg AE ha\(^{-1}\) in 1999 in plots \((2 \times 4 \text{ m}^2)\) established in each site. The times of application are listed in Table 1. Rates and time of application were those usually used by the farmers in accord with agricultural practices proposed by technical advisers. During 1997, one plot was established in each site. In 1998, three experimental plots were established in the site, each one with four replications and glyphosate was applied once, twice or three times (Table 1). During 1999, two plots were established in the site, each one with four replications, and glyphosate was applied once and twice.

In all the sites, soybean was directly seeded with a row distance of 50 cm. Treatments were applied by a mechanical sprayer with 100 litres ha\(^{-1}\) water.

2.2 Sample collection

Leaves, stems and grains were collected for residue analysis. Randomized sampling at each site was carried out two or three times at vegetative stage (leaves and stems) and once one week before harvest (leaves, stems and grains). In each plot, three groups of five plants were randomly selected and collected in plastic bags by cutting at a height of 5 cm with electric scissors. Samples of each group were placed separately in cold storage at \(-14^\circ\text{C}\) until analysed. In site 4, five plants were collected from each plot and were stored in the same way. During 1999, grain samples were not obtained at harvesting.

2.3 Sample analysis

All chemical analyses were performed by the Instituto de Tecnología (CONICET) in Santa Fe, Argentina. The method proposed by Fabre and Bordey was employed, modifying the EPA method using an ion exclusion column and a solution of phosphoric acid \((5 \text{ g litre}^{-1})\) as mobile phase. After elution from the analytical column, the glyphosate was oxidized with calcium hypochlorite and then coupled with the \(o\)-phthalaldehyde-2-mercaptoethanol complex at \(38^\circ\text{C}\) to give a compound detected by a scanning fluorescence detector (excitation at 340 nm and detection at 455 nm). Recovery was checked by adding glyphosate standard solution (corresponding to 1 mg kg\(^{-1}\), 5.0 mg kg\(^{-1}\) and 0.2 mg kg\(^{-1}\)) to 10-g blank samples. Mean recoveries (±SD) were 87 (±33), 83 (±58) and 113 (±28)% for 1, 0.5 and 0.2 mg kg\(^{-1}\), respectively. The method detection limit was 0.02 mg kg\(^{-1}\) and the quantitation limit was 0.15 mg kg\(^{-1}\). Plant samples were dried at 60\(^\circ\text{C}\) for 48 h, then ground and three sub-samples of 10 g were randomly taken for analyses. Samples were extracted by shaking with water + chloroform \((2 + 1\) by volume), according to the General Inspectorate for Health Protection. After centrifuging and decanting into a separating funnel, an aliquot of the supernatant was filtered through 2-µm membranes and analysed by high-performance liquid chromatography. The detection was made with a fluorescence detector after post-column derivatization.

2.4 Statistics

Residue levels obtained in 1998 were analysed by one-way ANOVA to test whether the number of glyphosate treatments led to different residue concentrations in plant samples. Least significant differences test was used for mean comparison when data were statistically significant.

| Table 1. Dates of soybean planting, timing of herbicide application, sampling dates and soybean harvest |
|---------------------------------------------------|------------------|------------------|------------------|
| Operation | Site 1–Site 2 | Site 3 | Site 4 | Site 5 |
| Plot 1 | Plot 2 | Plot 3 |
| Planting | November 30 | November 28 | November 28 | December 2 |
| Application | November 28 | November 28 | December 2 | December 2 |
| Sampling | December 10 | December 10 | December 10 | December 23 |
| Soybean harvest | April 7 | April 5 | April 8 | |

3 RESULTS

During 1997, glyphosate residues of 3.4–5.2 mg kg\(^{-1}\) were detected in soybeans after harvesting only in leaves and stems of plants treated twice with the herbicide. In the same organs, residues of AMPA were detected at higher values than glyphosate: 4.4–5.7 mg kg\(^{-1}\). Even when such residues are not statistically higher than those of glyphosate, their presence indicates metabolism of the herbicide. Grains of these plants contained very low residues of glyphosate, with a mean of 0.1–0.01 mg kg\(^{-1}\), and no AMPA residues were detected.

During 1998, glyphosate concentrations in leaves and stems of plants at harvesting stage differed according to the number of applications. Lower values were obtained with one spraying than in plots receiving two or three glyphosate treatments (Table 2). Residues were higher with two or three applications, when sprayings were applied 40–50 days before harvesting. AMPA residues were not detected in leaves or stems.

In 1999 glyphosate concentrations in soybean leaves and stems at harvesting stage were lower than in preceding years (Table 2) for one and two sprayings, even when rates were slightly higher. For two applications made later during the crop cycle, residue concentrations were higher than from applications performed earlier in the crop cycle, as was the case in 1997 and 1998 (Table 2).

In 1998, glyphosate and AMPA residues were detected in grains from soybean plants receiving two or three sprayings, but the difference between applications was not significant (Table 3). Glyphosate concentrations were higher than those obtained in 1997, but time of application was near harvesting in 1998 (Table 1). Residues in grains were much lower (40–50%) than those found in leaves and stems at harvesting (Table 3).

4 DISCUSSION

After application, glyphosate is absorbed by the foliage and translocated throughout stems, leaves and roots of the entire plant. In susceptible plants, the herbicide is neither broken down nor metabolized to a significant degree. The herbicidal action is the inhibition of EPSPS involved in aromatic amino-acid metabolism. Inhibition of amino-acid metabolism leads to a reduction of protein synthesis, resulting in cessation of growth, cellular disruption and plant death.\(^1\)

However, in RR soybeans, the enzyme EPSPS is not inhibited by the herbicide, so no damage is produced to the crop. It has been demonstrated that, after herbicide uptake, translocation to all parts of soybean plants occurs\(^1\) and AMPA is the main product of glyphosate metabolism in leaves. Glyphosate metabolism to AMPA in RR soybeans is consistent with its behaviour in conventional soybeans. We detected glyphosate residues in plant leaves and stems throughout the crop cycle from the vegetative stage to harvesting, levels being related to time of application. Concentrations were higher when sprayings were applied near harvesting, and no relationship with the application rate could be established. Glyphosate absorption and translocation in several crops and weed species during the 14-day period after application has been found to range from 3–38% of the amount applied.\(^17\) It might have happened that the period from the last spraying until harvest was too short for complete metabolism and dissipation of the herbicide in the plant. AMPA concentrations were different with different applications. Concentrations in grains appeared to be related to time of application.

Glyphosate is readily translocated and tends to concentrate in regions of high meristematic and metabolic activity.\(^1\) In glyphosate-resistant soybeans, residues of the herbicide and its metabolite AMPA were detected in grains, due to translocation and metabolism in the whole plant. Grain residues were also higher when applications were repeated and were closer to the reproductive stage. The present results demonstrate that glyphosate and AMPA residues exist in transgenic RR soybean.
So far, glyphosate has no known direct environmental impact on mammals, birds or fishes, and shows no bioaccumulation in the food chain. Introduction of glyphosate-resistant soybean is very recent (1996) and studies of residues evolution in this crop, as well as in oil and flour, should be done to evaluate human and animal dietary levels.

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REFERENCES